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A Malarial Parasite in the Blood of a Buffalo

BY

A. L. SHEATHER, B.Sc., M.R.C.V.S.,
Director and First Bacteriologist,
Imperial Bacteriological Laboratory, Muktesar

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A Malarial Parasite in the Blood of a Buffalo.

[*Received for publication on 19th March, 1919.*]

REFERENCE to the literature would appear to indicate that up to the present the occurrence of malarial parasites in the blood of ruminants has not been recorded except by Bruce, who found two antelopes infected in Nyasaland in 1913. This observation is published in the *Proceedings of the Royal Society,* Series B, Vol. 87, 1914. It is therefore desirable that a description, although incomplete, of a parasite found in an Indian buffalo should be placed on record.

Reference to Bruce's description of the parasite found by him in the duiker shews that that organism is morphologically quite distinct from the one described in this note.

The parasite was found in blood smears taken from a plains buffalo after death. Unfortunately it is impossible to do more than describe the morphology of the organism, as the animal died on the day fixed as a holiday to celebrate the signing of the armistice (Dec. 9th*) and the blood smears of the animals dying on that day were not examined until the following morning. As there were no grounds for suspecting any special infection, the carcase was destroyed on the day the animal died.

The buffalo was a male, four years old. It was bought in Bareilly in July 1918, and reached Muktesar early in August. After a period of quarantine it was taken into the sheds as a serum-maker. On August 23rd it was inoculated simultaneously with 0·5 c.c. of defibrinated virulent blood and 39 c.c. of anti-rinderpest serum (*i.e.,* 1 c.c. per 10 lb. body weight). The animal shewed a moderate temperature reaction but did not develop any other symptoms of rinderpest. The temperature fell to normal (about 38°) on the 11th day after inoculation, but it remained rather unsteady for several days. On September 12th the animal was bled for serum.

On September 14th it received its first hyper-immunizing dose of virulent blood. There was no distinct reaction, but there was a sudden rise and fall of temperature from 37·7° to 40° and back to 37·7° between the morning of the 22nd September and the morning of the 24th.

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* The lateness of this date is accounted for by the fact that paucity of European staff and isolation made it impossible to organize any celebration rapidly.

For ten days after this the temperature remained very steady, and the animal was bled for serum on the 24th and the 26th.

The temperature fell on the evening of October 4th to 37·2° and the following morning it was 36·8°. On the evening of October 5th it had again risen to 39·4° and it remained at about this level till the evening of October 10th.

The animal was again injected with virulent blood on October 9th.

The temperature remained normal, and the usual routine of injections and bleedings was carried out until November 8th. The animal had been bled on the morning of this day, and in the evening its temperature had risen to 40·3°. It had fallen to 39° on the following morning. On the evening of the 10th and the morning of the 11th there was another slight rise of temperature from 38·6° to 39·6°.

From this until December 4th, the temperature remained very steady, shewing slight rises only on the 15th, 25th and 26th November.

The animal was injected with virulent blood on November 23rd and bled for serum on December 3rd. From the evening of December 4th, when the temperature was 38·9°, there was a rapid fall until the morning of December 6th, when it was 36·1°. By the evening it had risen to 39·0°.

The animal was due for bleeding on this date, but as its temperature in the morning was so low, bleeding was not carried out.

On the morning of December 7th, the temperature was 38·8°, but during the day it fell rapidly, and by the following day it was 35°. The animal survived during the day but died during the night of December 8th.

From this it will be seen that there was no regular periodicity of fever.

Examination of the films shewed that there was present in the blood what appeared to be a complete series of types of parasite, in successive stages of development.

For the purposes of differential counting it was possible to divide them into three groups representing the most prominent members of the series. Forms intermediate between the three main groups were classified with that group to which they most closely approximated morphologically.

The three main types may be called :

(*a*) Small forms.
(*b*) Large forms.
(*c*) Dividing forms.

The immense majority of the parasites were intracorpuscular, but organisms belonging to each of three groups were found free in the plasma. For this there is a rational explanation which will be seen when the morphology of the parasites is described.

(*a*) *Small Parasites* * (Plate I, figs. 1 to 7). The smallest parasites found were distinctly piroplasm-like in appearance. They shewed a small rounded or slightly irregular piece of chromatin (occasionally two pieces) and a small amount of cell body, which in some appeared to be solid and in others to contain a vacuole. They varied somewhat in outline, but the majority were rounded, oval, or pear-shaped, and measured about 1 micron in their greatest diameter. Between these small parasites and the next group—the large parasites—there was a series of organisms of gradually increasing size. (Plate I, figs. 8 to 13.)

Apart, however, from the increase in size, there were other morphological details distinguishing them from the smallest organisms.

As the organisms increased in size, the number shewing a central vacuole rapidly increased and the vacuole itself became much larger. The increase in the size of the vacuole caused the chromatin to be pushed to one side.

Two pieces of chromatin could be distinguished in some of these organisms.

In organism measuring about 3 microns and upwards in diameter, a new morphological feature could be found in the presence of a small grain of deep brown pigment within the cytoplasm. From this stage up to the typical, large parasite, the parasites increased in size and the amount of pigment present was progressively larger.

(*b*) *Large Parasites* (Plate I, figs. 14 to 17) were for the most part circular in outline, but a few oval forms were found; the latter were possibly distorted in spreading the film.

The maximum diameter attained by the large parasites was about 6 microns. Organisms of this size differed somewhat among themselves in appearance.

In the majority of the large parasites there appeared to be a vacuole of considerable size, occupying a central position in the cytoplasm. In these the chromatin was pushed to one side and formed a crescent-shaped mass along the margin of the cell. In others the whole body of the parasite took the blue stain (Romanowsky) although the staining was almost invariably somewhat fainter in the centre than at the periphery.

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* All references to illustrations in the text refer to the coloured plate. A separate description is given for the photographs which are published in support of the drawings.

The chromatin was either in a single piece or in two pieces, and it was always somewhat loose in texture.

In these large parasites the pigment deposit was a striking feature. In some of the organisms, the granules were collected together into a single mass, and in others they were scattered through the cytoplasm. The individual granules were invariably rod-shaped, and gave the impression of being crystalline.

The next stage of development in these parasites appeared to be a preparation for division.

(*c*) *Dividing Forms* (Plate I, figs. 18 to 28). In parasites preparing to divide, the chromatin became spread out through the cell body in the form of fine interlacing filaments.

This was followed by a process of what may be termed condensation of the chromatin at a number of places among these filaments, leading to the production of a number of discrete chromatin particles embedded in the remains of the chromatin "web."

Dividing forms. These differed from the foregoing in that the interlacing filaments of chromatin had entirely disappeared, leaving only the discrete chromatin particles scattered through the cell body. These numbered from seven to fourteen. The next stage was the division of the cytoplasm into a number of pieces, each containing a fragment of nucleus. About the same time the enclosing red corpuscle appeared to have burst.

The small parasites thus formed were set free to invade other cells.

The pigment granules persisted throughout and were set free in the plasma with the new brood of parasites.

In a number of places in the film, leucocytes containing granules of pigment were found, and in a few instances leucocytes appeared to have ingested entire parasites.

Figs. 29 to 34 on Plate I shew some irregular forms of the parasite, and Fig. 35 shews pigment granules within a leucocyte.

Reference has already been made to the fact that parasites of all three types were found free in the plasma. The rupture of the corpuscle containing a parasite undergoing division is sufficient explanation for the presence of dividing forms and small parasites free in the plasma.

The presence of large forms free in the plasma is also explained by the rupture of the containing cells owing to the pressure put upon them by the presence of the parasite. Cells which contained large parasites were found measuring up to 14 microns in diameter, and it is reasonable to suppose that cells distended to this extent would be very liable to burst.

Multiple infection of cells. While in the majority of cases a single blood corpuscle contained a single parasite only, numerous examples were found of multiple invasion. These cases of multiple invasion were usually due to small parasites. Two, three, four and five small parasites were found in a single cell. This indicates that in such cases the multiple infection was actually multiple invasion and was not due to small parasites produced by division failing to escape; an additional point in favour of this view being that no pigment granules were found in such corpuscles.

There were also found cells containing two parasites of a size intermediate between the small and the large, and also cases in which a large and a small parasite occupied the same cell.

Extent of the blood invasion. An enumeration of the blood corpuscles shewed that 1·6 per cent. contained parasites.

Differential count of the parasites. As already stated, in making a differential count of the parasites, they were divided into three groups, (*a*) small, (*b*) large, and (*c*) dividing forms. The organisms intermediate between these types were apportioned to the group to which they most closely approximated. Thus, types between the small and large forms were assigned to one or other group, taking size and the presence of pigment into consideration.

In making the examination of the dividing forms, all parasites which shewed the preliminary formation of the chromatin "web" were considered as dividing forms. Working on these lines, it was found that the small forms numbered 35 per cent., the large forms 55 per cent., and dividing forms 10 per cent. of the whole number.

Changes in the blood. Apart from the presence of the parasite, the blood shewed many of the changes associated with anæmia, normoblasts, megaloblasts, anisocytosis, polychromasia, and punctate basophilia which were present to a marked degree.

It is possible that the changes in the blood-corpuscles may have been due to some extent to the withdrawal of 2,200 cubic centimetres of blood for serum on December 3rd.

Should the parasite here described prove to be a new one, I would suggest the name *Plasmodium bubalis* for it.

I am indebted to Babu Duni Chand, Artist on my staff, for the very accurate coloured drawings of the parasite and also for his assistance in obtaining the hoto-micrographs.

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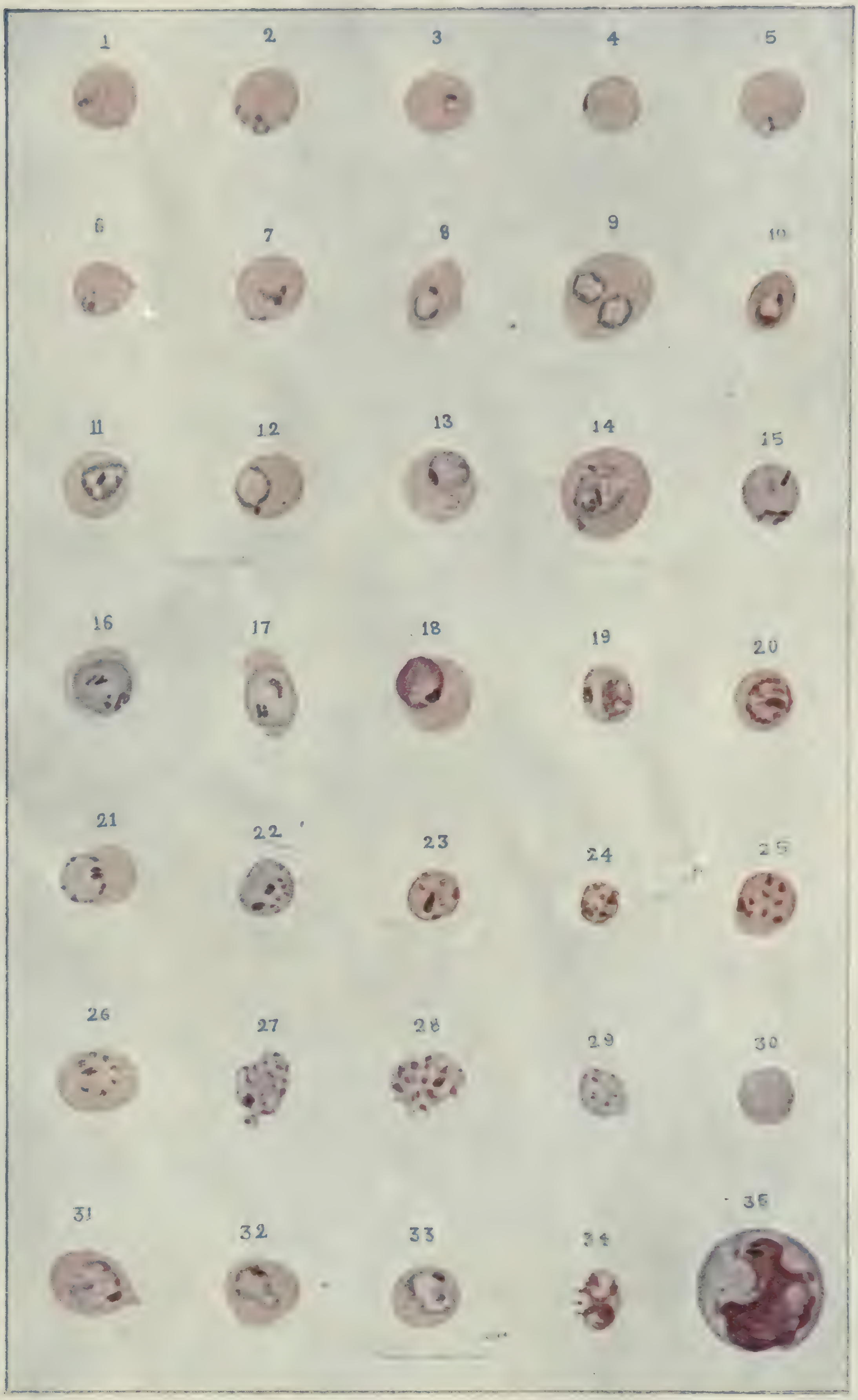
Description of Plate I.

- Figs. 1 to 7.—" Small " parasites.
  - Fig. 1.—Small parasite which has apparently just invaded the corpuscle.
  - Fig. 2.—Double invasion by small parasites. Vacuoles appearing in the cytoplasm.
  - Figs. 3 to 5.—Shewing the crescent-like shape of the chromatin.
  - Fig. 6.—Two distinct pieces of chromatin present in the parasite.
  - Fig. 7.—Double invasion by parasites of different ages. The larger shews small pigment granules.
- Figs. 8 to 13.—Parasites intermediate between small and large forms.
  - Fig. 8.—Parasite contains a large vacuole. Pigment and chromatin peripherally placed.
  - Fig. 9.—Double infection with two parasites of similar age.
  - Fig. 10.—Vacuolated parasite with pigment collected together in a single mass.
  - Fig. 11.—Pigment centrally placed and in the form of two distinct rods.
  - Fig. 13.—Double invasion of a blood corpuscle by an intermediate and a small parasite. The larger parasite is not vacuolated.
- Figs. 14 to 17.—Large parasites.
  - Fig. 14.—Double invasion of a blood corpuscle by a large and a small parasite. The large parasite shews the loose-textured chromatin and scattered pigment granules.
  - Fig. 15.—An extra corpuscular large parasite with loose-textured chromatin and scattered pigment granules, all of which are rod-shaped.
  - Fig. 16.—Closely resembles Fig. 15, but is intracorpuscular.
  - Fig. 17.—Large vacuolated parasite.
- Figs. 18 to 28.—Dividing forms.
  - Fig. 18.—The formation of the chromatin " web."
  - Fig. 19.—Commencing condensation of the chromatin.
  - Figs. 20 and 21.—Condensation of the chromatin nearly complete.
  - Figs. 22 to 26.—Various types of dividing parasites.
  - Figs. 27 & 28.—Dividing forms which have escaped from corpuscles and are on the point of splitting into a number of small parasites.
- Figs. 29 to 34.—Various irregular forms encountered in the specimen.
- Fig. 35.—Rod-shaped pigment granules enclosed in a leucocyte.



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A malarial parasite in the blood of a buffalo.

Description of Plate II.

Figs. 1 to 3.—Double and single invasions of corpuscles by small parasites.

Figs. 4 to 7.—Parasites intermediate between small and large forms.

Fig. 4.—Parasite not distinctly vacuolated.

Fig. 5.—Parasite distinctly vacuolated. Chromatin and pigment disposed at the periphery.

Fig. 6.—Double invasion by two intermediate parasites.

Fig. 7.—Double invasion by a small and an intermediate parasite.

Figs. 8 to 14.—Large parasites.

Fig. 8.—Double invasion by a large and a small parasite.

The large parasite is distinctly vacuolated.

The pigment is collected into an oval mass at one side and the chromatin forms a crescent-shaped mass on the other.

Fig. 9.—Closely resembles Fig. 8.

Fig. 11.—A large parasite shewing a bundle of pigment rods and a single mass of rather loose-textured chromatin.

Fig. 12.—Large non-vacuolated parasite with two rounded masses of loose chromatin and a number of scattered pigment rods.

Fig. 13.—Closely resembles Fig. 12, but the two masses of chromatin are more distinctly visible.

Fig. 14.—Large extra corpuscular parasite.

Fig. 15.—Four large parasites in a single field. The uppermost resembles Fig. 13, and the second and lowermost resembles Fig. 11. The remaining one which is a little distorted shews the first stage in the process of division. The chromatin is beginning to spread through the cytoplasm to form the chromatin " web."

Figs. 16 to 20.—Dividing parasites.

Figs. 16 & 17.—Shew the chromatin spreading out in the form of interlacing filaments.

Fig. 18.—The process of condensation of the chromatin has started.

Three small parasites are present in the corpuscle at the same time. (These are not quite in focus.)

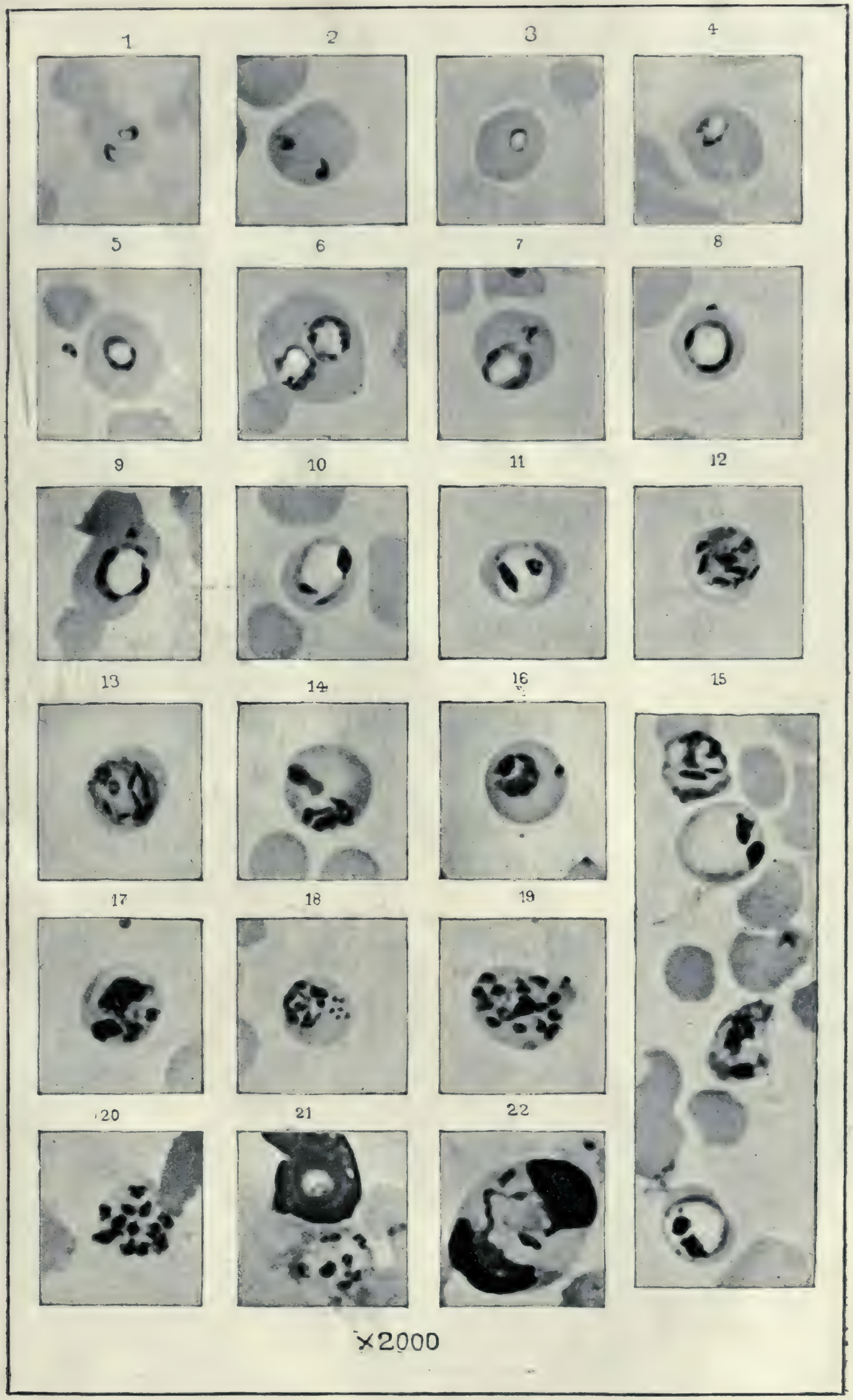
Fig. 19.—A parasite ready to fragment but still enclosed in a red corpuscle.

Fig. 20.—A parasite free from its corpuscle becoming fragmented.

Fig. 21.—The remains of a dividing parasite which has been ingested by a leucocyte.

Fig. 22.—Pigment granules within a leucocyte.

PLATE II



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